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GB 1381932
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(54) **Method for producing purine arabinosides**

(57) A 9-(β-D-arabinofuranosyl) purine which is optionally substituted at the 2,6- and/or 8-position can be produced by reacting an arabinose donor with a purine source in the presence of an enzyme capable of effecting transarabinosylation.

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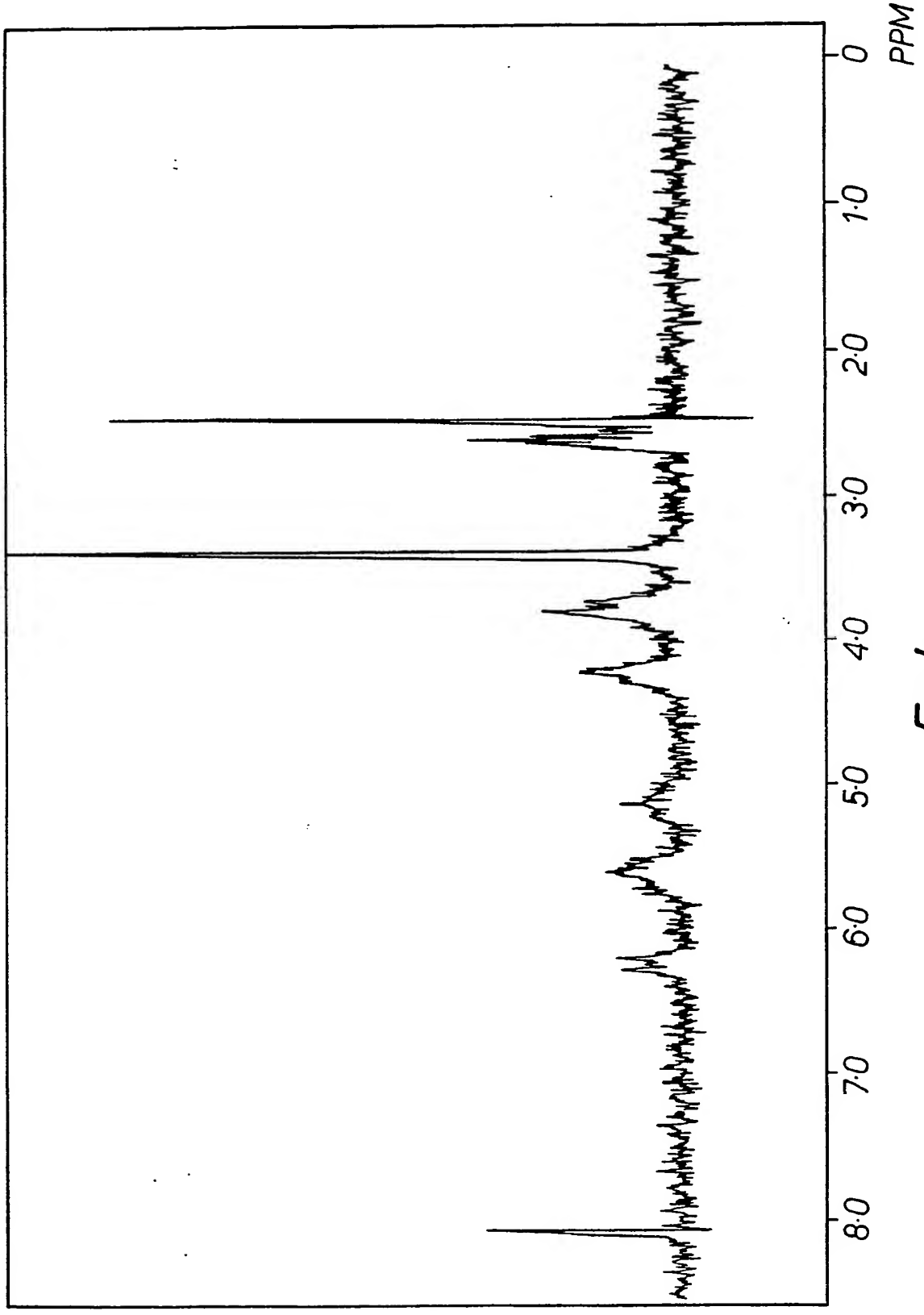

FIG. 1.

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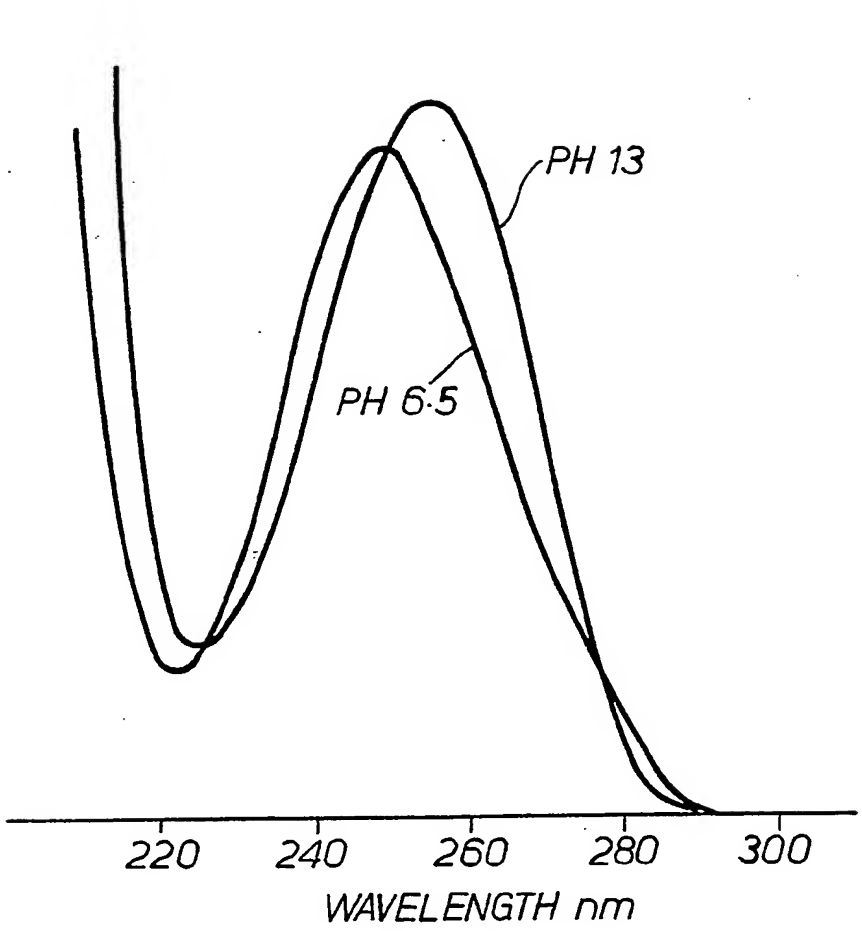

FIG.2.

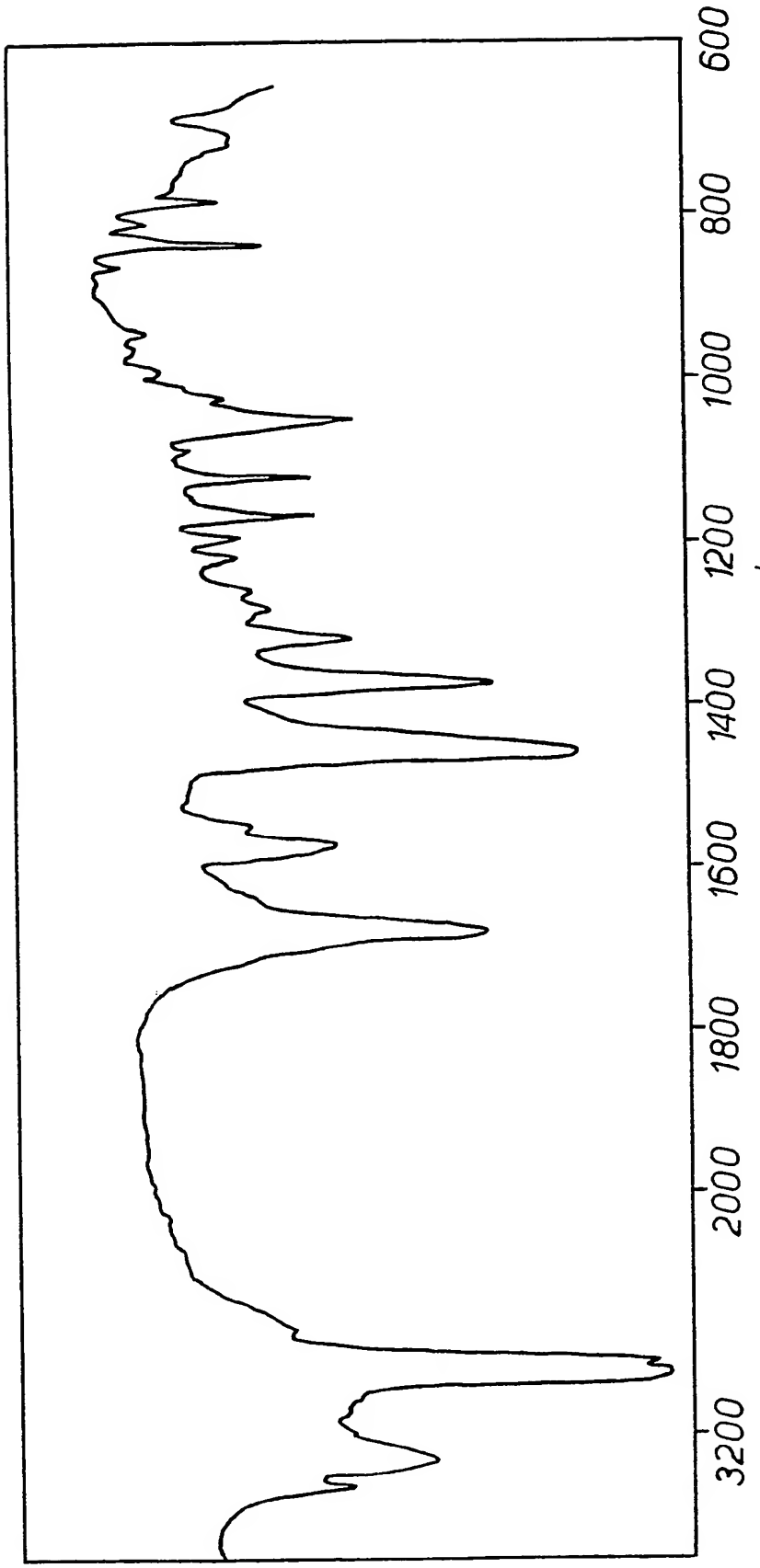

FIG.3.

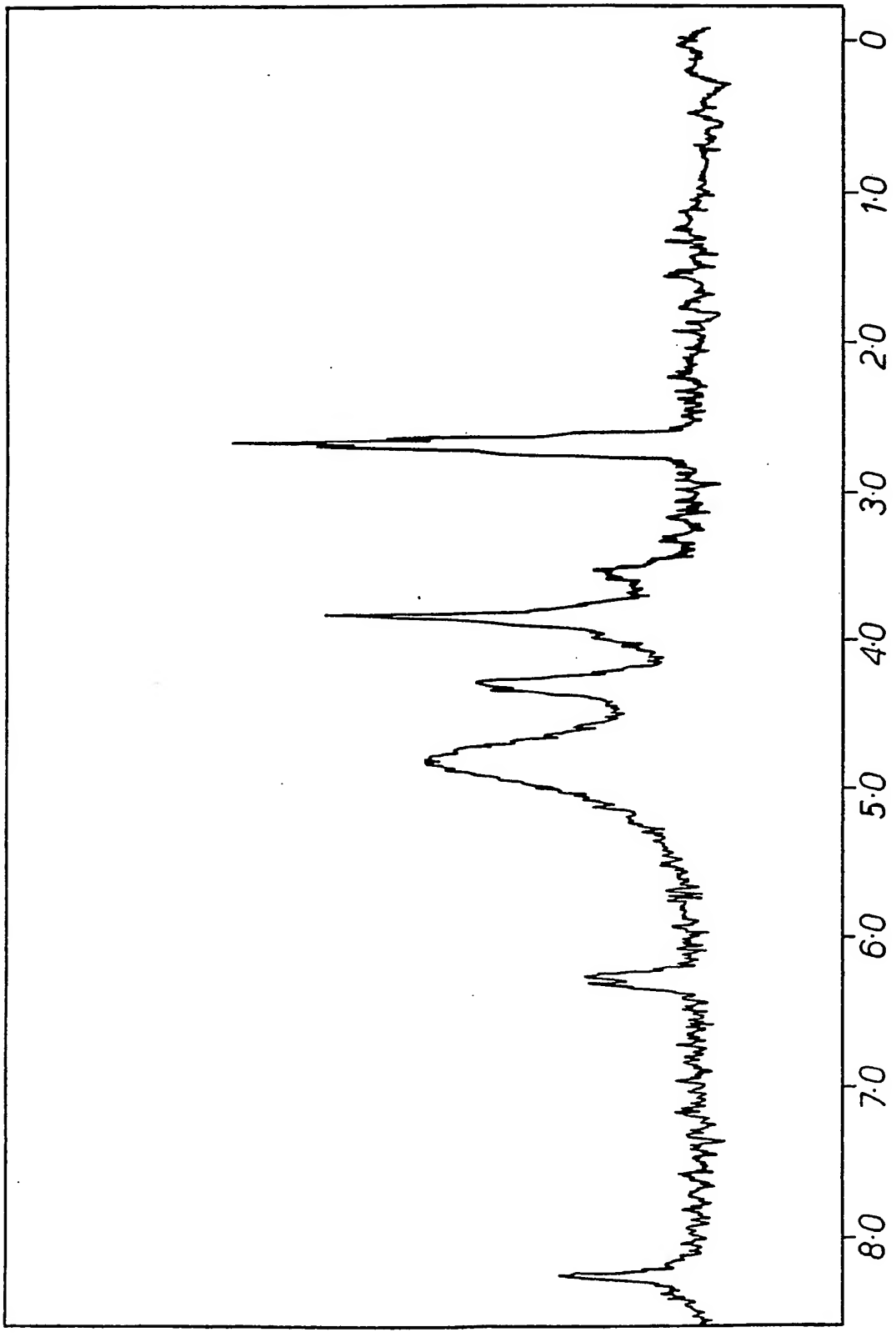

FIG.4.

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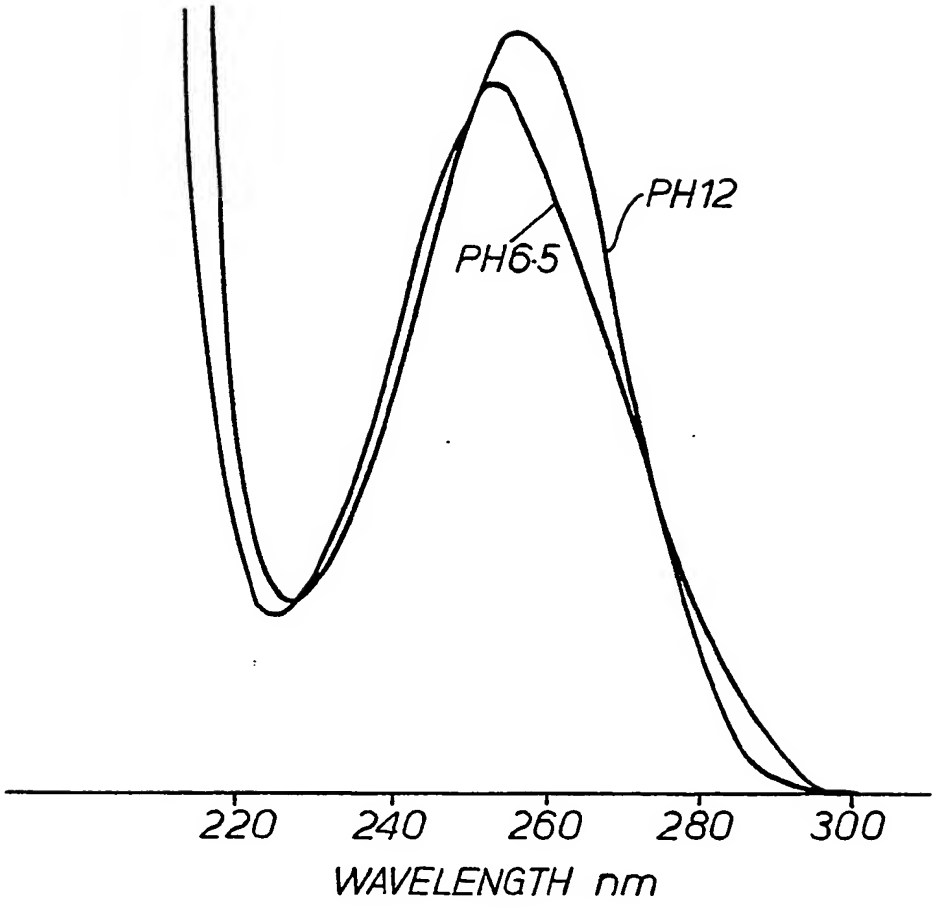

FIG. 5.

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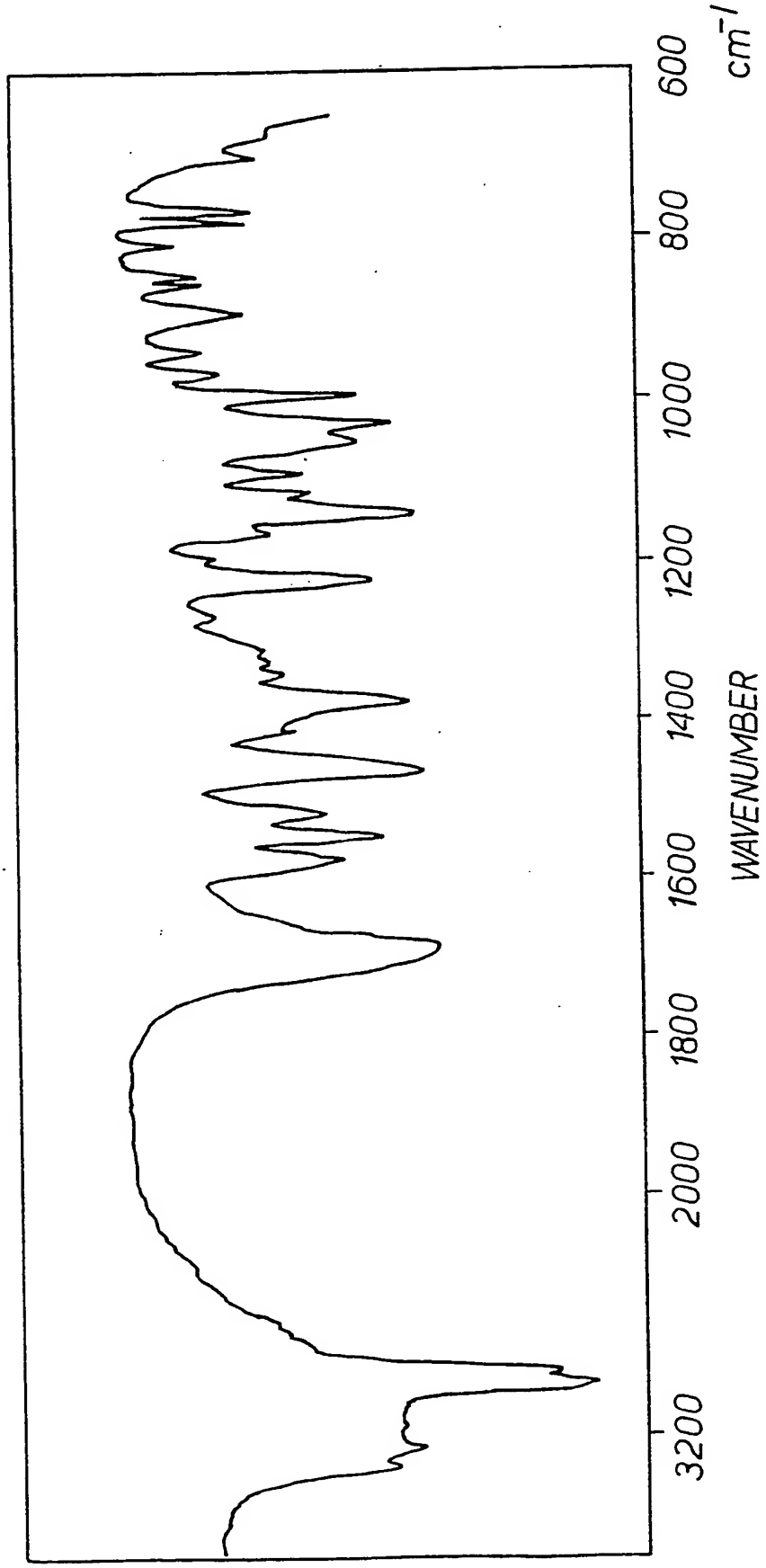


FIG.6.

SPECIFICATION

Method for producing purine arabinosides

This invention relates to a method for producing purine-arabinosides, particularly by an enzymatic process.

Purine-arabinosides (9-(β-D-arabinofuranosyl)-purines) have potential utility as agricultural chemicals or medicinal agents. For example, it has been reported that adenine arabinoside, one of the purine arabinosides, has been used successfully to treat several diseases caused by the herpes virus including chickenpox and shingles.

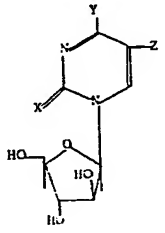
As to known methods for producing the purine arabinosides, several chemically synthetic methods have been proposed, (J. Org. Chem. *27*, 3274, (1962); J. Org. Chem. *28*, 3004 (1963); J. Org. Chem, *32*, (1976); Tetrahedron Letters 1970, 4673; and Japanese Published Examined Patent Application No. 7271/1972). It is further reported that adenine arabinoside is produced when Streptomyces antibiotics is cultured in conventional culture media (Japanese Published Examined Patent Application No. 41558/1972).

It has been found that purine arabinosides are produced in aqueous reaction media from arabinose donor such as uracil arabinoside or D-arabinofuranose-1-phosphate and purine-source such as adenine, hypoxanthine, adenosine and adenosine-5-monophosphate by the action of an enzyme produced by various bacteria.

Now, commercially applicable method for producing purine arabinosides has been provided by

(a) holding at the temperature of the range from 40° to 70°C in an aqueous medium an arabinose donor selected from the group consisting of D-arabinofuranose-1-phosphate and the compound having Formula 1 and the nucleotide of the compound, and a purine source selected from the group consisting of unsubstituted or 2,6 and/or 8-substituted purine and its ribofuranoside, ribofuranotide, deoxyribofuranoside or deoxyribofuranotide, in the presence of an effective amount of enzyme produced by the bacterium and capable of transarabinosylation from the arabinose donor to the unsubstituted or 2,6 and/or 8-substituted purine of the purine source, whereby β-D-arabinofuranosyl radical is attached to the 9-position of the unsubstituted or 2,6 and/or 8-substituted purine; and

(b) recovering the produced 9-(β-D-arabinofuranosyl)- unsubstituted or 2,6 and/or 8-substituted purine.



X represents O, S or NH;

Y represents OH, NH_2, SH or SR(R is lower alkyl group); and

Z represents H, Halogen, NO_2, CH_3 or CH_2OH.

The arabinose donors of this invention are D-arabino furanose 1 phosphate, the compound showing formula I, or the phosphate of the compound showing formula I. The specimens of the arabinose donors are shown in Examples of this invention.

The purine sources of this invention are unsubstituted or 2,6 and/or 8-substituted purine and its ribofuranoside, deoxgribofuranoside or deoxyribofuranotide. 2,6 and/or 8-substituted purine used in this invention as the purine source can be detected by the following method: Ribofurano-side of 2,6 and/or 8-substituted purine is held with the enzyme of this invention in an aqueous medium containing 0.1 M KH_2PO_4 at 60°C for 24 hours. When 2,6 and/or 8-substituted purine of the originally used 2,6 and/or 8-substituted purine ribofuranoside, and D-ribofuranose 1 phosphate or D-ribose derived from the above ribofuranoside are produced in the aqueous medium, the 2,6 and/or 8-substituted purine can be used as the purine source.

The substituents of 2,6 and/or 8-substituted purines are, for example, halogen, hydroxyl, amino, lower alkyl, alkoxyl, aryl, aralkyl, mercapto, alkylamino alkylmercapto, alkysulfonyl, alkylsulfenyl, carboxyl, alkoxycarbonyl, cyano, and nitro radicals.

The D-arabinofuranose of the arabinose donor is enzymatically transferred to and attached to 9-position of the ubsubstituted or 2,6/or 8-substituted purine of the purine source. Thus, the product of this invention is 9-(β-D-arabinofuranosyl)-unsubstituted or 2,6 and/or 8-substituted purine.

The bacterial enzyme capable of transarabinosylation from the arabinose donor to unsubstituted or 2,6 and/or 8-substituted purine of the purine source is produced mainly in the bacterial cells and its present to a small extent in the supernatant of the culture liquids. The bacteria capable of producing the enzyme belong, found so far, to the genera Pseudomonas, Flavobacterium, Achromobacter, Salmonella, Citrobacter, Escherichia, Klebsiella, Enterobacter, Aeromonas, Serratiam, Erwinia, Proteus, Xanthomonas, and Bacterium.

Specimens of the bacteria are:
Pseudomonas stutzeri NRRL B-11346 (FERM-P 4170),
Flavobacterium rhenanum NRRL B-11343 (CCM 298),
Flavobacterium acidoficum ATCC 8366,
Flavobacterium proteus ATCC 12841,
Achromobacter lacticum NRLL B-11340 (CCM 69),
Salmonella typhimurum NRLL B-11347 (FERM-P3735),
Citrobacter freundii ATCC 8090,
Citrobacter freundii ATCC 6750
(Citrobacter intermedium)
Escherichia coli ATCC 9637,
Escherichia aurescens ATCC 12814,
Klebsiella pneumoniae ATCC 9621,
(Enterobacter aerogenes
Serratia Liquefaciens ATCC 14460,
(Enterobacter liquefaciens)
Enterobacter aerogenes ATCC 13048,
Aeromonas punctata ATCC 11163,
Aeromonas salmonicida ATCC 14174,
Serratia marcescens IFO 3048,
Erwinia carotovora NRRL B-11342 (CCM 872),
Erwinia amylovara NRRL B-11341 (CCM 1017),
Erwinia herbicola ATCC 14537,
Proteus vulgaris NRRL B-11345 (FERM-P3394),
Proteus rettgeri NRRL B-11344 (FERM-P3395),
Bacterium cadaveris IFO 3731, and
Xanthomonas citri NRRL B-11348 (FERM-P3396).

In order to produce the enzyme using the bacteria as mentioned above, the bacteria are cultured in or on conventional culture media. The culture media contain conventional carbon sources, nitrogen sources, inorganic ions, and when required minor organic nutrients such as vitamines and amino acides. Usual manner can be applied to culture the bacteria in the conventional media, that is, the bacteria are cultured aerobically preferably at a pH of a range from 4 to 9 and a temperature of a range from 25 to 40°C.

As the enzyme source, intact cells, culture liquids containing the cells are used preferably. Additionally, cells dried with acetone, freeze-dried cells, homogenated cells, cells treated with super sonic waves, cells treated with toluene, surfactants or lysozyme are employed giving desirable results. Moreover protein fractions having the enzyme activity capable of transarabinosylation from the arabinose donor to unsubstituted or 2,6 and/or 8-substituted purine of the purine source can be used preferably as the enzyme source. It is expected that there are more than one enzyme participating in the production of the purine arabinosides.

The production of the purine arabinosides can be carried out by holding in the culture media of the bacteria the purine source and the arabinose donor. In this case, the arabinose donor and purine source are added into the culture media after the bacteria sufficiently grew, and thereafter the temperature is maintained at 40°C to 70°C. The production of the purine arabinoside can be also carried out by contacting the purine source and arabinose-donor with the cells or the enzyme sources as mentioned above in aqueous reaction media other than culture media. Thus, in this invention, "aqueous medium" means culture medium or reaction medium (reaction mixture). The reaction media are maintained preferably at a temperature from 40°C to 70°C, and at a pH of 4 to 10 for 5 to 100 hours.

The reaction temperatyre (40°C to 70°C) of this invention is specific in the point that the temperature is higher than the ordinarly enzyme reaction temperature, and critical.

The purine arabinosides produced in the culture media or the reaction media can be recovered by conventional manners such as ion exchange method or crystallization technique.

Example 1

An aqueous culture medium of pH 7.2 was prepared which contained, per deciliter, 0.5g yeast extract, 1.0g peptone, 0.5g bouillon, and 0.5g NaC1. Five ml batches of the aqueous culture medium were placed in test tubes, and heated to sterilize. Each one loopful inoculum of the bacteria listed in Table 1 was transferred into each batch of the aqueous culture medium. Cultivation was carried out at 30°C for 36 hours with shaking. The cells produced in the cultire liquid were collected by centrifugation and washed with physiological saline. The cells thus obtained (50mg(wet)/ml) were suspended in samples of 0.05M phosphate buffer of ph7.0, and 0.5ml of the suspension of the cells was mixed with 0.5ml of reaction mixture of ph7.5 containing 0.5g/dl uracil arabinoside, 0.2g/dl hypoxanthine and 50mg/dl KH_2PO_4. Each mixture was held at 60°C for 15 hours, and thereafter heated to 100°C for 5 minutes.

Each product in the reaction mixture was identified as 9-β-D-arabinofuranosyl hypoxanthine (hypoxanthine arabinoside) by high speed liquid chromatography, and the amounts of the hypoxanthine arabinoside in the reaction mixture were determined by the high speed liquid chromatography, and are shown in Table 1.

Table 1

microorganism used		hypoxanthine arabinoside accumulated mg/dl
NRRL	B-11343	3.7
ATCC	8366	6.6
ATCC	12841	6.7
NRRL	B-11340	5.7
NRRL	B-11347	7.5
ATCC	8090	11.3
ATCC	6750	13.2
ATCC	9637	10.5
ATCC	12814	17.0
ATCC	9621	126.0
ATCC	14460	17.0
ATCC	14174	36.0
ATCC	11163	4.1
IFO	3048	23.0
NRRL	B-11342	14.0
NRRL	B-11341	18.0
ATCC	14537	21.0
NRRL	B-11345	9.6
NRRL	B-11344	2.4
NRRL	B-11348	11.0
IFO	3731	12.0
NRRL	B-11346	7.5
ATCC	13048	55.7

Example 2

In the method shown in Example 1, adenine was substituted for hypoxanthine, and the amounts of adenine arabinoside shown in Table 2 were produced in the reaction mixture.

Example 3

In the method shown in Example 1, cytosine arabinoside was substituted for uracil arabinoside and the amounts of hypoxanthine arabinoside shown in Table 3 were produced in the reaction mixture.

Example 4

In the method shown in Example 1, adenine riboside-5-monophospate was substituted for hypoxanthine, and the amounts of adenine arabinoside shown in Table 4 were accumulated in the reaction mixture.

Table 2

microorganism used	adenine arabinoside accumulated mg/dl
NRRL B-11343	4.5
ATCC 8366	8.2
ATCC 12841	8.0
NRRL B-11340	6.5
NRRL B-11347	8.6
ATCC 8090	13.3
ATCC 6750	15.0
ATCC 9637	10.6
ATCC 12814	18.8
ATCC 9621	132.0
ATCC 14460	26.0
ATCC 14174	41.0
ATCC 11163	18.5
IFO 3048	32.6
NRRL B-11342	20.5
NRRL B-11341	22.5
ATCC 14537	31.5
NRRL B-11345	26.3
NRRL B-11344	28.6
NRRL B-11348	13.5
IFO 3731	21.2
NRRL B-11346	8.6
ATCC 13048	71.8

Table 3

microorganism used	hypoxanthine arabinoside accumulated mg/dl
NRRL B-11343	4.2
ATCC 8366	5.5
ATCC 12841	8.2
NRRL B-11340	2.6
NRRL B-11347	4.8
ATCC 8090	6.5
ATCC 6750	10.3
ATCC 9637	6.3
ATCC 12814	3.6
ATCC 9621	82.1
ATCC 14460	15.0
ATCC 14174	20.5
ATCC 11163	0.8
IFO 3048	13.6
NRRL B-11342	2.6
NRRL B-11341	8.7
ATCC 14537	15.0
NRRL B-11345	8.1
NRRL B-11344	0.5
NRRL B-11348	0.8
IFO 3731	10.6
NRRL B-11346	3.2
ATCC 13048	40.2

Table 4

microorganism used		adenine arabinoside accumulated mg/dl
NRRL	B-11343	3.8
ATCC	8366	5.6
ATCC	12841	7.2
NRRL	B-11340	3.5
NRRL	B-11347	8.3
ATCC	8090	10.2
ATCC	6750	8.6
ATCC	9637	5.5
ATCC	12814	6.9
ATCC	9621	82.3
ATCC	14460	13.5
ATCC	14174	25.5
ATCC	11163	9.6
IFO	3048	21.5
NRRL	B-11342	15.5
NRRL	B-11341	11.5
ATCC	14537	18.3
NRRL	B-11345	12.6
NRRL	B-11344	15.8
NRRL	B-11348	8.3
IFO	3731	14.5
NRRL	B-11346	8.5
ATCC	13048	49.6

Example 5

A hundred ml batches of the aqueous culture medium shown in Example 1 were placed in 500ml shaking flask and heated to sterilize. Klebsiella pneumoniae ATCC 9621 was inoculated in the aqueous culture medium and cultured at 30°C for 36 hours with shaking. Cells produced in the resultant culture liquid were collected by centrifugation, and 30g (wet) of the cells was put into 1l of the reaction mixture of ph 7.0 containing 1.5g 2-methylhypoxanthine, 7.3g uracil arabinoside and 3.4g KH_2PO_4. The reaction mixture was held at 60°C for 36 hours.

Cells were removed from the reaction mixture by centrifugation, the supernatant was passed through cation exchange resin ("Amerlite CG-120"), (Registered Trade Mark), and the resin was washed with 0.1N ammonium acetate (ph 6.8). After eluting with 0.1N ammonium hydroxide, the eluate was evaporated and cooled, and 710mg crystals were obtained.

The crystalline product was determined as 9-(β-D-arabinofuranosyl)-2-methylhypoxanthine (2-methylhypoxanthine arabinoside) by NMR spectrum, UV spectrum, IR spectrum, and elemental analysis.

Elemental analysis:

calculated; C : 46.8%, H : 5.0%, N : 19.8%
found; C : 46.5%, H : 5.1%, N : 19.5%

NMR spectrum : shown in Figure 1.
UV spectrum : shown in Figure 2.
IR spectrum : shown in Figure 3.

Example 6

Thirty grams of the cells obtained in Example 4 was put into 1ℓ of reaction mixture containing 1.7g 2-chloro hypoxanthine, 7.3g uracil arabinoside, and 3.4g KH_2PO_4, and the reaction mixture was held at 60°C for 36 hours. After removing the cells from the reaction mixture, the supernatant was passed through anion exchange resin (*"Dowex* IX4") (Registered Trade Mark), and the resin was washed with 0.1N ammonium acetate of pH6.8. After eluting with 0.1N ammonium acetate of pH4.0, the eluate was evaporated, and charged on *"Sephadex* G-10" (Registered Trade Mark) and developed with water. The eluate portions showing first peal of UV absorption of the two were collected, evaporated and cooled. Then, 326mg crystals were obtained.

The crystalline product was determined at 9-(β-D-arabinofuranosyl)-2-chlorohypoxanthine(2-chlorohypoxanthine arabinoside) by NMR spectrum, UV spectrum, IR spectrum, elemental analysis and Beilstein test.

Elemental analysis:

calculated;	C : 39.68,	H : 3.66, N : 18.51
found;	C : 39.42,	H : 3.72, N : 18.25

NMR spectrum : shown in Figure 4.

UV spectrum : shown in Figure 5.

IR spectrum : shown in Figure.6.

Beilstein test : positive (green)

Example 7

In the method shown in Example 1, 2-methylhypoxanthine or 2-chlorohypoxanthine was substituted for hypoxanthine, and the amounts of 2-methylhypoxanthine arabinoside or 2-chlorohypoxanthine arabinoside shown in Table 5 were accumulated in the reaction mixture.

Example 8

In the method shown in Example 1, 0.2g/dl hypoxanthine was replaced with 0.4g/dl inosine, and the amounts of hypoxanthine arabinoside shown in Table 6 were produced in the reaction mixture.

Example 9

A hundred ml of the aqueous culture medium shown in Example 1 was placed in a 500ml shaking flask, heated to sterilize, and inoculated with Aeromonas salmonicida ATCC 14174. Cultivation was carried out at 30°C for 36 hours with shaking.

Cells produced in the resultant culture liquid were collected by centrifiguation, and 2.0g (west weight) of the cells were put into 100ml reaction mixture of ph 7.5 contakining 100mg hypoxanthine, 300mg uracil arabinoside and 50mg KH_2PO_4. The reaction mixture was then held at 60°C for 15 hours.

Twenty five mg crystals of hypoxanthine arabinoside were obtained from the reaction mixture.

Table 5

microorganism	2-methylhypoxanthine arabinoside accumulated mg/dl	2-chlorohypoxanthine arabinoside accumulated mg/dl
NRRL B-11343	2.1	0.5
ATCC 8366	3.4	0.8
ATCC 12841	4.0	2.1
NRRL B-11340	5.5	2.5
NRRL B-11347	4.8	2.8
ATCC 8090	8.7	3.6
ATCC 6750	9.5	8.2
ATCC 9637	4.7	5.1
ATCC 12814	12.0	10.5
ATCC 9621	80.5	51.6
ATCC 14460	18.5	11.3
ATCC 14174	21.6	10.0
ATCC 11163	0.8	0.05
IFO 3048	15.4	10.8
NRRL B-11342	2.4	0.1
NRRL B-11341	12.0	10.5
ATCC 14537	15.5	12.1
NRRL B-11345	0.6	0.05
NRRL B-11344	8.2	0.5
NRRL B-11348	12.5	0.8
IFO 3731	21.6	2.1
NRRL B-11346	15.3	10.3
ATCC 13048	40.2	28.7

Table 6

microorganism used		hypoxanthine arabinoside accumulated mg/dl
NRRL	B-11343	2.8
ATCC	8366	3.6
ATCC	12841	5.5
NRRL	B-11340	4.3
NRRL	B-11347	6.2
ATCC	8090	8.8
ATCC	6750	7.4
ATCC	9637	1.6
ATCC	12814	13.6
ATCC	9621	83.3
ATCC	14460	6.2
ATCC	14174	16.8
ATCC	11163	0.9
IFO	3048	15.3
NRRL	B-11342	6.8
NRRL	B-11341	10.2
ATCC	14537	8.9
NRRL	B-11345	8.5
NRRL	B-11344	0.8
NRRL	B-11348	7.2
IFO	3731	5.8
NRRL	B-11346	3.3
ATCC	13048	40.4

Example 10

Klebsiella pneumonias ATCC 9621 was cultured by the manner shown in Example 9. Cells in the resultant culture liquid were collected by centrifugation, and 2 g (wet weight) of the cells were suspended in 100ml reaction mixture of pH7.5 containing 100mg hypoxanthine, 300mg cytosine arabinoside, 50mg KH_2PO_4, and the reaction mixture was held at 60°C for 15 hours.

The cells in the reaction mixture were removed by centrifugation, and a concentrate of the supernatant was passed through anion exchange resin ("Dowex-1" OH form, pH6.8). After eluting with 0.1N formic acid of pH4.0, the eluate was passed through "Sephadex G-10". Eluate (250ml) obtained by eluting with water was concentrated and the concentrate was added with methanol and cooled to form crystals of the products. After re-crystallization with water, 35mg purified crystals were obtained.

The crystalline product was idenitified with authentic hypoxanthine arabinoside by NMR spectrum, IR spectrum and UV spectrum.

Example 11

Klebsiella pneumonias ATCC 9621 was cultured by the same manner as in Example 9, and cells were collected by centrifugation.

Hypoxanthine in the reaction mixture in Example 9 was replaced with adenine, and the reaction mixture was held at 60°C for 15 hours. The supernatant of the reaction mixture was concentrated to 20 ml. Upon cooling the concentrate, 80 mg crystals were obtained.

The crystalline product was identified with authentic adenine arabinoside with NMR spectrum, IR spectrum, and UV spectrum.

Example 12

Erwinia hervicola ATCC 14537 was cultured by the same manner as in Example 9, and the cells produced were collected by centrifugation.

The cells thus obtained (2g (wet weight)/dl) were suspended in 100ml of a reaction mixture of pH7.5 containing 100mg/dl adenine, 300mg/dl cytosine arabinoside and 50mg KH_2PO_4, and held at 60°C for 15 hours.

After removing the cells from the reaction mixture, the reaction mixture was concentrated at 20ml, and cooled. The crystals thus obtained were recrystallized with water and 55mg purified crystals were obtained. The crystalline product was identified with adenine arabinoside by NMR spectrum, IR spectrum and UV spectrum.

Example 13

Cells (5g (wet)/dl) of Aeromonas salmonicida ATCC 14174 were suspended in 100ml batches of a reaction mixture containing 30mM cytosine arabinoside, 25mM KH_2PO_4, and 10mM of one of the purines shown in Table 7. The reaction mixtures were placed in test tubes and held at 60°C for 15 hours.

Newly formed product having UV absorption in the resaltant reaction mixture was separated by liquid chromatography. The eluate of the chromatography was concentrated and added with ethanol, whereby crystals were formed in the eluate.

From NMR spectra and UV spectra of the purified crystalline products, the products were ascertained as the arabinosides of the respective purines used as the starting materials.

Conversion ration of the purine arabinosides from purine source were determined by measuring molecular exteinction co-efficient, and are shown in Table 7.

Table 7

Starting material	Product	Conversion ratio (%)
xanthine	xanthine arabinoside	15
guanine	guanine arabinoside	8
purine	purine arabinoside	23
6-mercaptopurine	6-mercaptopurine arabinoside	8
2,6-diaminopurine	2,6-diaminopurine arabinoside	38
6-mercaptoguanine	6-mereaptoguanine arabinoside	7
2-methylhypoxanthine	2-methyhpoxanthine arabinoside	35
2-chlorohypoxanthine	2-chlorohypoxanthine arabinoside	18

Example 14

Cells (5g (wet)/dl) of Klebsiella pneumonias ATCC 9621 were suspended in 100ml batches of a reaction mixture placed in test tubes, containing 30mM uracil arabinoside, 25mM KH_2PO_4, and one of the purine sources (10 mM) listed in Table 8, and the reaction mixture was held at 60°C for 15 hours.

Newly formed product having UV-absorption in the resultant reaction mixture was separated by liquid chromatography. The eluate of the chromatography was concentrated and added with ethanol, whereby crystals were formed in the eluate.

From NMR spectra of the purified crystalline products, the products were ascertained as the arabinoside of the respective purine sources used as starting materials.

Conversion rate of purine arabinosides from the purine sources used was determined by measuring molecular extinction coefficient, and are shown in Table 8

Table 8

Starting material	Product	Conversion ratio (%)
xanthine	xanthine arabinoside	65
guanine	guanine arabinoside	20
purine	purine arabinoside	36
6-mercaptopurine	6-mercaptopurine arabinoside	8
2,6-diaminopurine	2,6-diaminopurine arabinoside	52
6-mercaptoguanine	6-mercaptoguanine arabinoside	5

Example 15

In the method shown in Example 5, 2-methylhypoxanthine was replaced with 2-ethylhypoxanthine. The resultant reaction mixture was charged on silica-gel thin-layer, and the chromatogram was developed with water-saturated butanol. The part of Rf 0.4 having absorption at 260 nm on the thin-layer was collected, and suspended in 0.1NHC1, and silicagel was removed from the suspension.

When the supernatant of the suspension was made 6N with HC1 and boiled for 10 minutes, orcinol-ferric chloride reaction of the boiled suspension became positive, and 2-ethylhypoxanthine was found in the boiled suspension by paper-chromatography. Thus, it is suggested that 2-ethylhypoxanthine arabinoside was produced in the reaction mixture.

Example 16

Cells of Klebsiella pneumoniae ATCC 9621 were obtained by the same manner as in Example 9, suspended in 0.5M phosphate buffer of pH 7.5 to obtain 100 g (wet)/1, and treated with super sonic waves.

A hundred ml of a reaction mixture, of pH 7.5 containing 50 ml/dl the supernatant, 500mg/dl uracil arabinoside-5-monophosphate, 100 mg/dl hypozanthine and 30 mg/dl KH_2PO_4, was held at 60°C for 15 hours. Then the reaction mixture was centrifuged to remove precipitates, and the supernatant was passed through cation exchange resin ("Chromobead C-2").

Elution was made with 0.3N formic acid, and the eluate was charged on anion exchange resin ("Dowex 1 × 4"). Hypoxanthine arabinoside was eluted by gradient elution with ammonium formate of pH 9 to 3 and 8 mg of crystals were obtained from the eluate.

Example 17

One ml of a reaction mixture containing, per milliliter, 0.2 ml of the supernatant shown in Example 16, 10 mg uracil arabinoside, 2 mg KH_2PO_4, 2 mg of one of the purine sources shown below was held at 60°C in a test tube for 15 hours, and heated at 100°C for 5 minutes.

After removing precipitates in the reaction mixture the reaction mixture was subjected to paper chromatograph, and the spot having UV-absorption and having a Rf value different from that of the purine sources used as the starting material was cutted, and put into 0.1N HC1. Then the 0.1N NC1 was made 6N by adding concentrated HC1 after removing filter paper, and boiled for 10 minutes, arabinose was found by orcinol-ferric chloride reaction in the boiled 6NHC1. Thus, it is expected that arabinosides of the purine sources used as the starting materials were produced in the reaction mixtures

6-chloropurine 6-mercaptopurine
2-chlorohypoxanthine 6-methylthiopurine
2-aminopurine 2-amino-6-mercaptopurine
2-methylthiohypoxanthine 6-carboxypurine
8-chloroadenine 8-bromoadenine

Example 18

In the method shown in Example 16. uracil arabinoside-5'-monophosphate was replaced with cytocine arabinoside-5'-monophosphate. In the resultant reaction mixture, hypoxanthine arabinoside was found.

Table 9

microorganism used	hypoxanthine arabinoside accumulated mg/dl
NRRL B-11343	2.8
ATCC 8366	5.5
ATCC 12841	6.3
NRRL B-11340	6.0
NRRL B-11347	5.2
ATCC 8090	8.8
ATCC 6750	10.6
ATCC 9637	8.5
ATCC 12814	12.3
ATCC 9621	103.6
ATCC 14460	12.5
ATCC 14174	29.3
ATCC 11163	4.0
IFO 3048	24.0
NRRL B-11342	15.2
NRRL B-11341	17.6
ATCC 14537	22.3
NRRL B-11345	15.6
NRRL B-11344	3.2
NRRL B-11348	10.6
IFO 3731	18.3
NRRL B-11346	8.2
ATCC 13048	48.5

Example 19

In the method shown in Example 1, D-arabinofurnose-1-phosphate was substituted for uracil arabinoside, and the amounts of hypoxanthine arabinoside shown in Table 9 were accumulated in the reaction mixture.

Example 20

In the method shown in Example 19, one of the purine sources listed in Table 10 was substituted for hypoxanthine, and newly formed product having UV-absorption in the resultant reaction mixture was separated by preparative high speed liquid chromatography. The eluate of the chromatograph was concentrated and added with ethanol, whereby crystals were formed in the eluate. From NMR spectra and UV spectra of the crystalline products, the products were ascertained as the arabinosides of the respective purine sources used as the starting materials.

Conversion ratio of the purine sources used to the purine arabinosides was determined by measuring molecular extention coefficient and shown in Table 10.

Table 10

Starting material	product	Conversion ratio (%)
xanthine	xanthine arabinoside	15
guanine	guanine arabinoside	8
purine	purine arabinoside	23
6-mercaptopurine	6-mercaptopurine arabinoside	8
2,6-diaminopurine	2,6-diaminopurine arabinoside	38
6-mercaptoguanine	6-mercaptoguanine arabinoside	7
2-methylhypoxanthine	2-methylhypoxanthine arabinoside	35
2-chlorohypoxanthine	2-chlorohypoxanthine arabinoside	18

Example 21

Klebsiella pneumonias ATCC 9621 was cultured by the same manner as in Example 9, and the cells produced were collected by centrifugation.

20 mg of the cells obtained were suspended in 1 ml of a reaction mixture of pH 7.0 containing 1.5 mg of adenine. 10 mg of one of the pyrimidine arabinosides listed in Table 11, and 3.4 mg of KH_2PO_4, and the reaction mixture was held at 60°C for 15 hours.

Cells were removed from the reaction mixture by centrifugation. Adenine arabinoside accumulated was identified by high speed liquid chromatography.

Table 11

arabinose donor
4-thiouracil arabinofuranoside
4- (S-methyl-)thiouracil arabinofuranoside
2-thiouracil arabinofuranoside
5-nitrouracil arabinofuranoside
5-hydroxymethyluracil arabinofuranoside
isocytosine arabinofuranoside
5-fluorouracil arabinofuranoside
5-bromouracil arabinofuranoside
5-Iodouracil arabinofuranoside
thymine arabinofuranoside

Example 22

In the method shown in Example 11, adenine in the reaction mixture was replaced with 200 mg adenylic acid, and the reaction mixture was held at 60°C for 15 hours. The supernatant of the reaction mixture was concentrated to 30 ml. Upon cooling the concentrate, 48 mg crystals were obtained. The crystalline product was idenitified with authentic adenine arabinoside with NMR spectrum IR spectrum, and UV spectrum.

Example 23

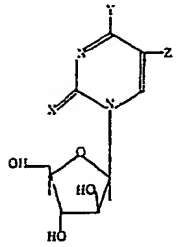
In the method shown in Example 11, adenine in the reaction mixture was replaced with 150 mg guanosine, and the reaction micture was held at 60°C for 15 hours. The crystals of 2-(β-D-arabinofuranosyl)guanine (guanine arabinoside) (28mg) were obtained from the supernatant of the resulted reaction mixture.

Example 24

In the method shown in Example 13, adenosine, deoxyadenosine, deoxyadenylic acid, guanylic acid, deoxyguanylic acid, xanthosine, deoxyxanthosine, deoxyinosine or deoxyinosinic acid were used in place of hypoxanthine as the purine source. From the above adenine source, adenine arabinoside was formed in the reaction mixture and separated by the usual manner. From the above guanine source, guanine arabinoside was formed. From the above xanthine source, the xanthine arabinoside was formed. From the hypoxanthine source, hypoxanthine arabinoside was formed.

CLAIMS:

1. A process for producing 9-(β-D-arabinofuranosyl) purine which is optionally substituted in the 2,6- and/or 8-position, which process comprises holding at a temperature in the range from 40°C to 70°C in an aqueous medium an arabinose donor selected from D-arabinofuranose-1-phosphate, a compound having the formula I (set out below) and in the phosphate of that compound, and a purine source selected from an unsubstited or 2,6-and/or 8-substituted purine and its ribofuranoside, ribofuranotide, deoxyribofuranoside and deoxyribofuranotide, in the presence of an effective amount of an enzyme produced by a bacterium and capable of transarabinosylation from the arabinose donor to the unsubstituted or 2,6- and/or 8-substituted purine of the purine source, whereby the β-D-arabinofuranosyl radical is attached to the 9-position of the unsubstituted or 2,6- and/or 8-substituted purine; wherein formula I is as follows:



in which X represents O, S or NH, Y represents OH, NH_2, SH or SR where R is lower alkyl group, and Z represents a hydrogen or halogen aton or NO_2, CH_3 or CH_2OH.

2. A process according to claim 1, wherein the substitutent(s) of 2,6- and/or 8-substituted purine is/are selected from halogen, hydroxyl, amino, lower alkyl, alkoxyl, aryl, aralkyl, mercapto, alkylamino, alkylmercapto, alkysulphonyl, alkysulphenyl, carboxyl, alkoxycarbonyl, nitro and cyano radicals.

3. A process according to claim 1, wherein the substitutent(s) of 2,6- and/or 8-substituted purine is/are selected from amino, hydroxyl and mercapto radicals.

4. A process according to claim 1,2 or 3, the purine source is adenine, adenosine, deoxyadenosine, deoxyadenylic acid, adenylic acid, hypoxanthine, inosine, deoxyinosine, deoxyinosinic acid, inosinic acid, guanine, guanosine, deoxyguanosine, deoxyguanylic acid, guanylic acid, xanthine, xanthosine, deoxyxanthosine or xanthylic acid.

5. A process according to any preceding claim, wherein the arabinose donor is 1-β-D-arabinofuranosyl-cystosine or 1-β-D-arabinofuranosyl-uracil.

6. A process according to any preceding claim, wherein the bacterium belongs to the genus *Pseudomonas, Flavobacterium, Achromobacter, Salmonella, Seratis, Aeromonas, Erwinia, Prateus, Bacterium, Xanthomonas, Klebsiella* or *Citrobacter.*

7. A process according to any one of claims 1 to 5, wherein the bacterium belongs to the genus *Escherichia.*

8. A process according to any one of claims 1 to 5, wherein the bacterium belongs to the genus *Enterobacter.*

9. A process according to any one of claims 1 to 5, wherein the bacterium is
Pseudomonas stutzeri NRRL B-11346,
Flavobacterium rhenanum NRRL B-11343,
Flavobacterium acidoficum ATCC 8366,
Flavobacterium proteus ATCC 12841,
Achromobacter lacticum NRRL B-11340,
Salmonella typhimurum NRRL B-11347,
Citrobacter freundii ATCC 98090,
Citrobacter freundii ATCC 6750
Klebsiella pneumoniae ATCC 9621,
Serratia liquefaciens ATCC 14460,
Aeromonas punctata ATCC 11163,
Erwinia carotovora NRRL B-11342,
Erwinia amylovara NRRL B-11341,
Erwinia herbicola ATCC 14537,
Proteus vulgaris NRRL B-11345,
Proteus rettgeri NRRL B-11344,
Bacterium cadaveris IFO 3731, or
Xanthomonas citri NRRL B-11348.

10. A process according to any one of claims 1 to 5, wherein the bacterium is *Escherichia Coli* ATCC 9637, *Escherichia aurescens* ATCC 12814, or *Enterobacter aerogenes* ATCC 13048.

11. A process according to any preceding claim, wherein the pH of the aqueous medium is in the range from 4 to 10.

12. A process according to any preceding claim, wherein cells of the bacterium are used as the enzyme.

13. A process according to any preceding claim, which includes recovering the resulting 9-(β-D-arabinofuranosyl) purine which is optionally substituted at the 2,6- and/or 8-position, from the aqueous medium.

14. A process according to claim 1, substantially as described in any one of the foregoing Examples.

15. A 9-(β-D-arabinofuranosyl) purine which is optionally substituted at the 2,6 - and/or 8-position and which is produced by a process accofrding to any preceeding claim.

16. 9-(β-D-arabinofuranosyl)-2-chlorohypoxanthine.

17. 9-(β-D-arabinofuranosyl)-2-methylhypoxanthine.

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